Supporting Information

An effective polymeric nanocarrier that allows for active targeting and selective drug delivery in cell coculture systems

*Maria Victoria Cano-Cortes[†,a,b,c], Patricia Altea Manzano[†,a,d], Jose Antonio Laz-Ruiz[†,a,b,c], Juan Diego Unciti-Broceta[e,] Francisco Javier Lopez-Delgado[a,f], Jose Manuel Espejo-Roman[a,b,c], Juan Jose Diaz-Mochon[*a,b,c] and Rosario M. Sanchez-Martin[*a,b,c]*

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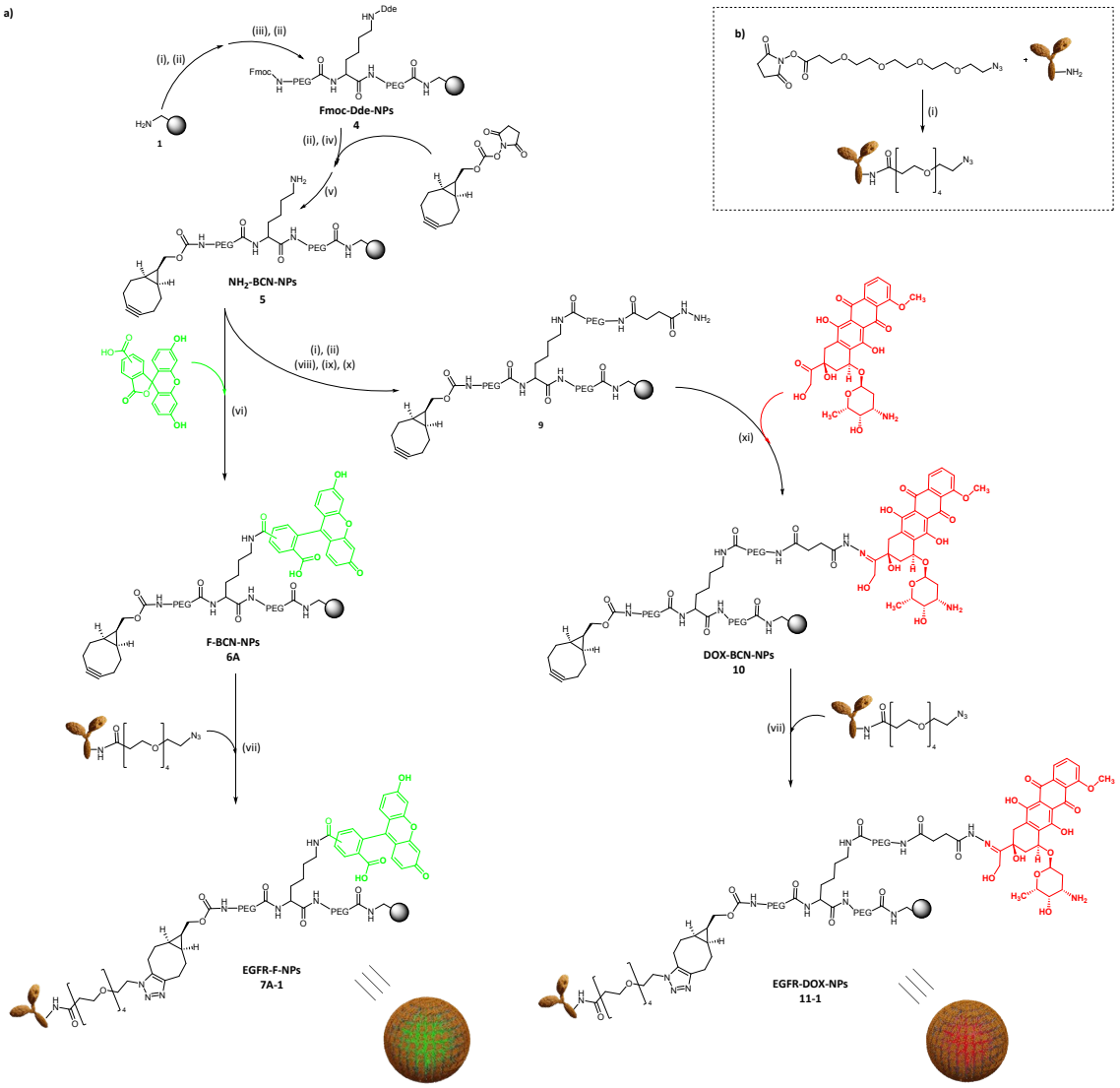


Scheme S1. General scheme of synthesis of EGFR-NPs. Reagents and conditions. (i) Fmoc-PEG-OH (15 eq), Oxyma (15 eq), DIC (15 eq), DMF, 2 h, 60 °C; (ii) 20% piperidine/DMF, 3 x 20 min; (iii) Fmoc-Lys-Dde(OH) (15 eq), Oxyma (15 eq), DIC (15 eq), DMF, 2 h, 60 °C; (iv) BCN-NHS (10 eq), DIPEA (1 eq), DMF, 15 h, 25 ºC; (v) Hydroxylamine.HCl, Imidazole, NMP, 2 x 1 h, 25 ºC; (vi) 5(6)-carboxyfluorescein (15 eq), Oxyma (15 eq), DIC (15 eq), DMF, 2 h, 60 °C; (vii) Ab-NHS-N3, PBS, 15h, 25ºC; (viii) Succinic anhydride (15 eq), DIPEA (15 eq), 2 h, 60 °C; (ix) Oxyma (15 eq), DIC (15 eq), 2 h, 25 °C; (x) Hydrated hydrazine 55% v/v (15 eq), 15 h, 25 °C; (xi) Doxorubicin (1 eq), PBS pH 6, 15 h, 50 ° C. b) (i) NHS-N_3 1mM, PBS, anti-EGFR, 2h, 4 ºC.

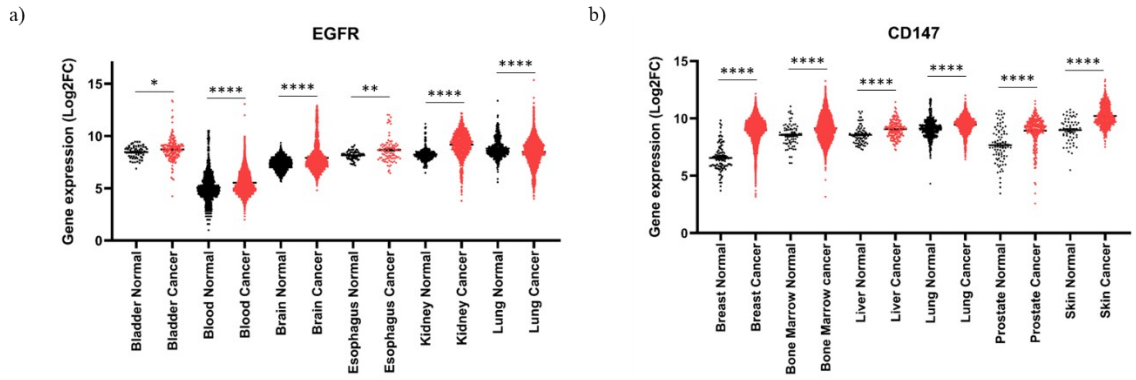


Figure S1. Surface markers in tumors based on gene expression profiles across diverse human cancer and normal tissues. [1] a) EGFR and b) CD147.

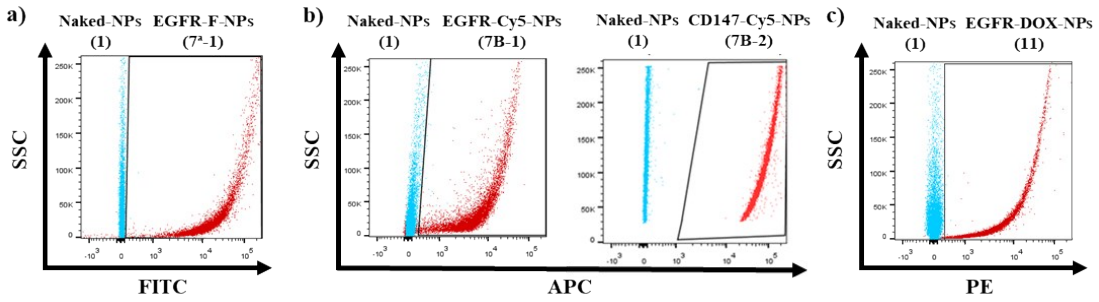
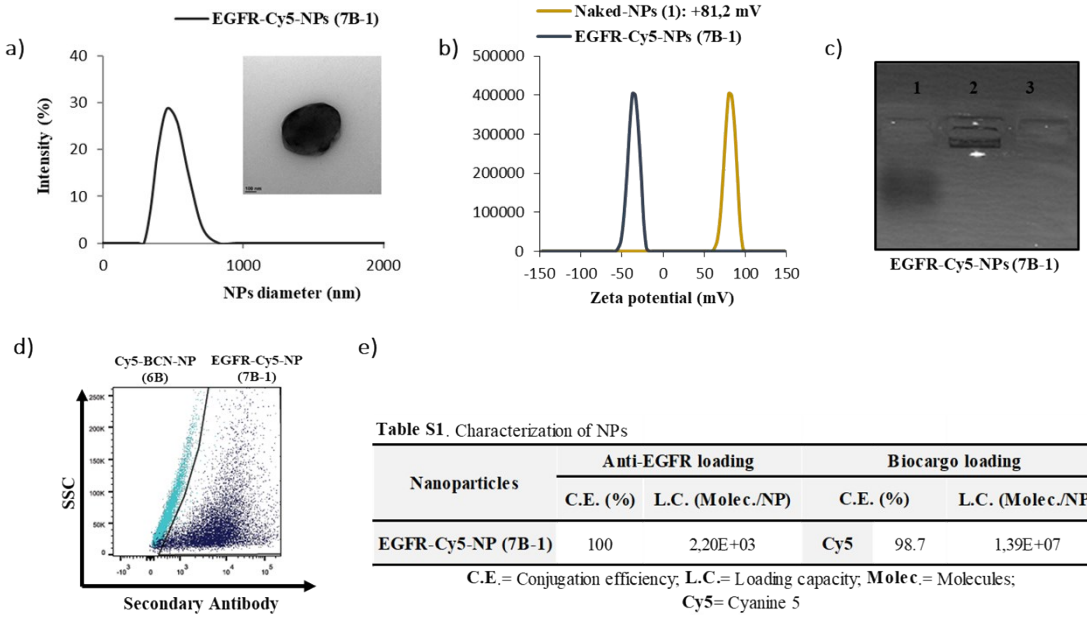


Figure S2. Dot plots representative of flow cytometry of Naked-NPs (**1**) (blue) and a) EGFR-F-NPs (**7A-1**) (red); b) EGFR-Cy5-NPs (**7B-1**) and CD147-Cy5-NPs (**7B-2**) (red); c) EGFR-DOX-NPs (**11-1**) (red).



e)

Table S1. Characterization of NPs

Nanoparticles	Anti-EGFR loading		Biocargo loading	
	C.E. (%)	L.C. (Molec./NP)	C.E. (%)	L.C. (Molec./NP)
EGFR-Cy5-NP (7B-1)	100	2,20E+03	Cy5	98.7	1,39E+07

C.E.= Conjugation efficiency; **L.C.**= Loading capacity; **Molec.**= Molecules;
Cy5= Cyanine 5

Figure S3. Characterization of EGFR-Cy5-NPs (**7B-1**): a) Hydrodynamic diameter values by DLS and TEM image; b) Zeta potential value; c) Coomassie staining of gel electrophoresis; d) Immunofluorescence of EGFR-Cy5-NPs (**7B-1**) versus Cy5-BCN-NPs (**6B**); e) Determination of conjugation efficiency and loading capacity of anti-EGFR and Cy5 per nanoparticle.

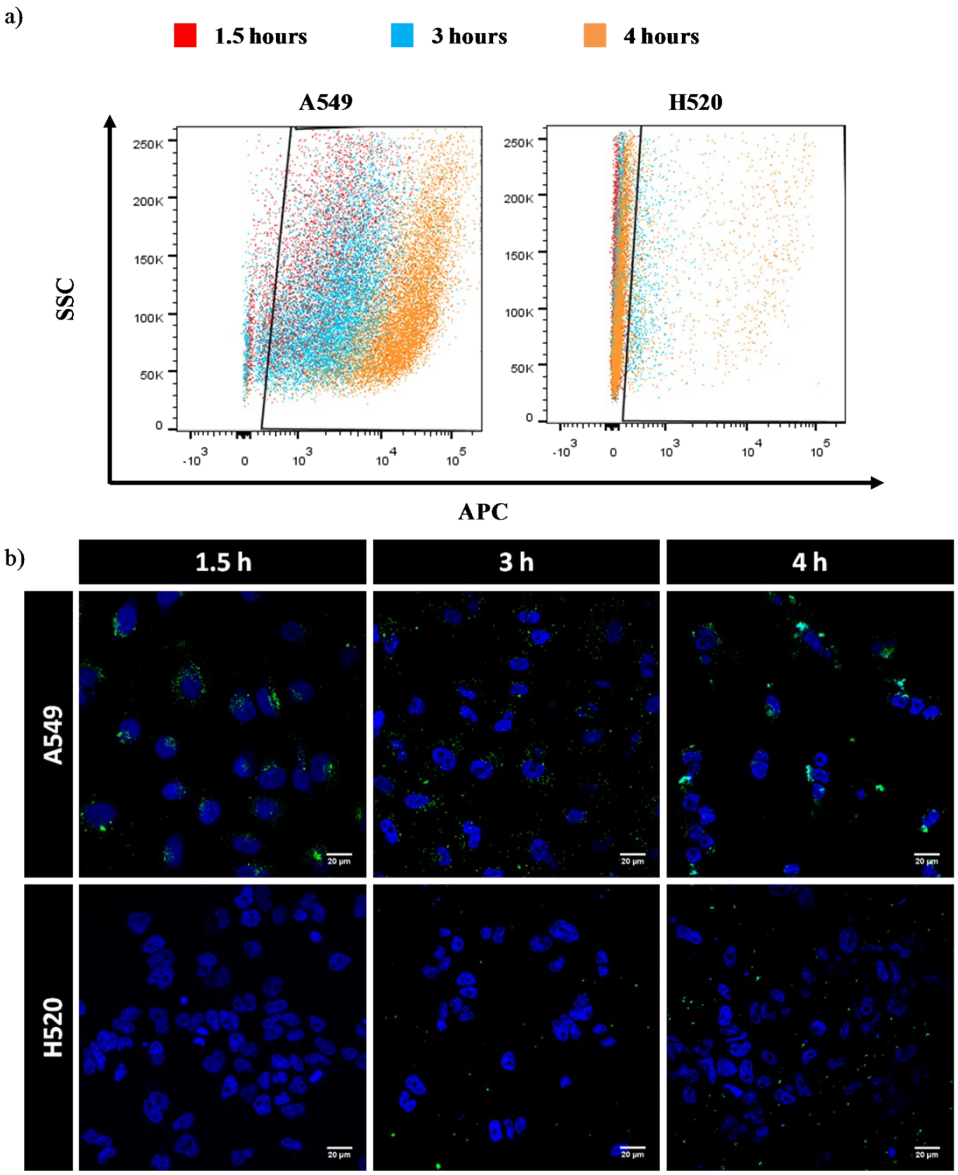


Figure S4. Analysis of nanofection of EGFR-F-NPs (**7A-1**) at different time points . a) Flow cytometry analysis: A549 (high) and H520 (low) cell lines were incubated with 1,000 NPs per cell for 1.5 h (red), 3 h (blue) and 4 h (orange) for nanofection experiments. b) confocal microscopy: Confocal microscope images of the cellular uptake behavior in A549 and H520 cell lines. 40X magnification representative images show: merge, blue, DAPI nuclei and green, EGFR-F-NPs (**7A-1**) overlaid. Scale bar, 20 µm.

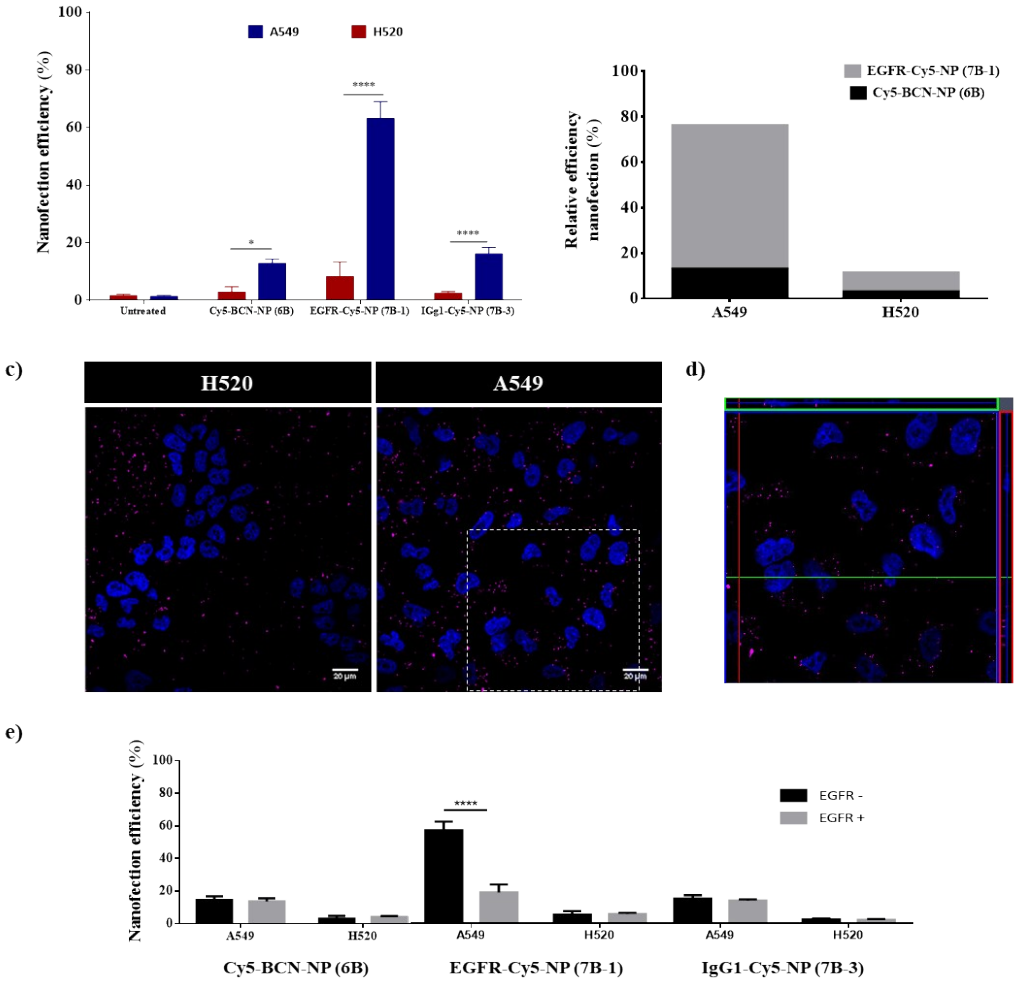


Figure S5. Selective nanofection and viability assays of EGFR-Cy5-NPs (**7B-1**). The A549 (high) and H520 (low) cell lines were incubated with 1,000 NPs per cell for 1.5 h for nanofection experiments and the results were analyzed by flow cytometry; a) Comparison of the efficacy of nanofection of EGFR-Cy5-NPs (**7B-1**) against the controls used; b) Uptake of EGFR-Cy5-NPs (**7B-1**) related to uptake of NPs prior to conjugation with the antibody (Cy5-BCN-NPs (**6B**)); c) Confocal microscopy of the cellular uptake behavior of EGFR-Cy5-NPs (**7B-1**); d) Orthogonal view (xy, xz, and yz) of the confocal microscope images showing the intersection planes at the position of the cross-line. Maximum intensity projection of the z-stack from blue (DAPI, nuclei) and magenta for EGFR-Cy5-NPs (**7B-1**) in A549 cell line is displayed. e) Competitive binding assay.

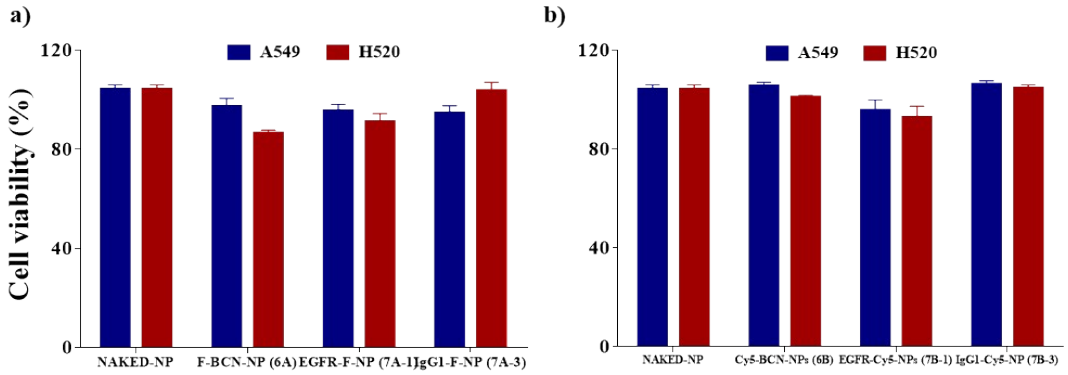


Figure S6. Viability of A549/H520 cell lines by resazurin cell viability assay after the internalization of a) F-BCN-NP (**6A**). EGFR-F-NP (**7A-1**) and IgG1-F-NP (**7A-3**); b) Cy5-BCN-NPs (**6B**), EGFR-Cy5-NP (**7B-1**) and IgG1-Cy5-NPs (**7B-3**), compared to Naked-NPs.

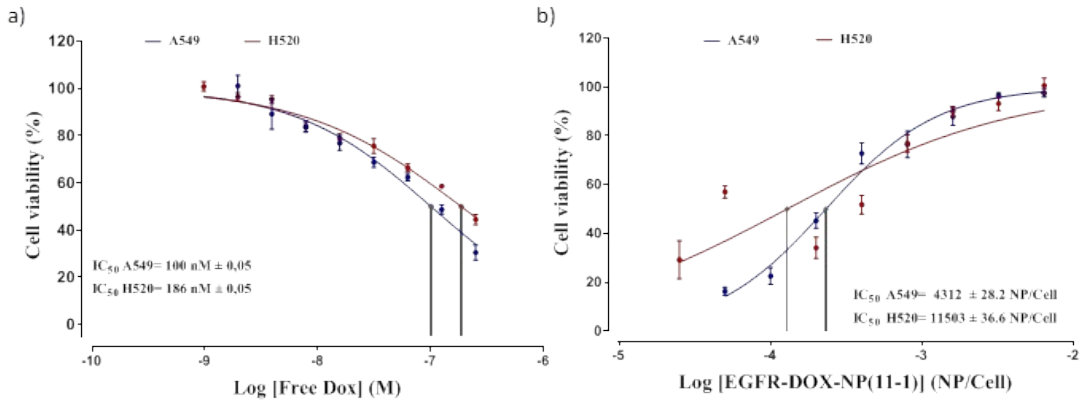
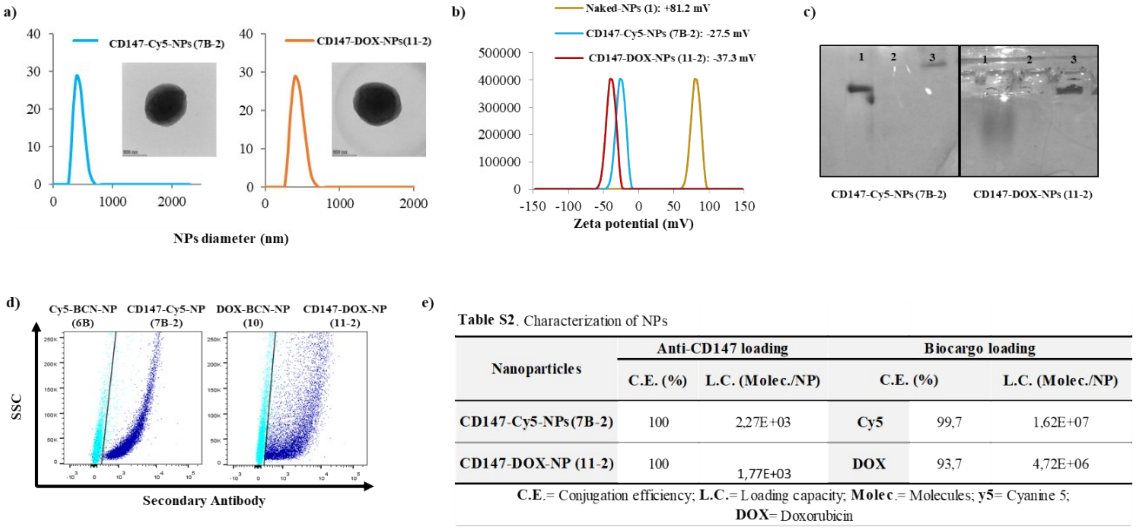


Figure S7. Dose-response curves (percentage of cell viability versus concentration) of treatment of A549 and H520 cancer cells with: a) free DOX, represented in M; b) EGFR-DOX-NPs (**11-1**), represented in NPs/Cell. The IC50 value was determined by the logarithm (inhibitor) versus normalized response: variable slope using the GraphPad software.



e)

Table S2. Characterization of NPs

Nanoparticles	Anti-CD147 loading		Biocargo loading		
	C.E. (%)	L.C. (Molec./NP)	C.E. (%)		L.C. (Molec./NP)
CD147-Cy5-NPs(7B-2)	100	2,27E+03	**Cy5**	99,7	1,62E+07
CD147-DOX-NP (11-2)	100	1,77E+03	**DOX**	93,7	4,72E+06

C.E.= Conjugation efficiency; **L.C.**= Loading capacity; **Molec**.= Molecules; **y5**= Cyanine 5; **DOX**= Doxorubicin

Figure S8. Characterization of CD147-Cy5-NPs (**7B-2**): a) Hydrodynamic diameter values by DLS and TEM image; b) Zeta potential value; c) Coomassie staining of gel electrophoresis; d) Immunofluorescence of CD147-Cy5-NPs (**7B-2**) versus Cy5-BCN-NPs (**6B**); e) Determination of conjugation efficiency and loading capacity of anti-CD147 and Cy5 per nanoparticle.

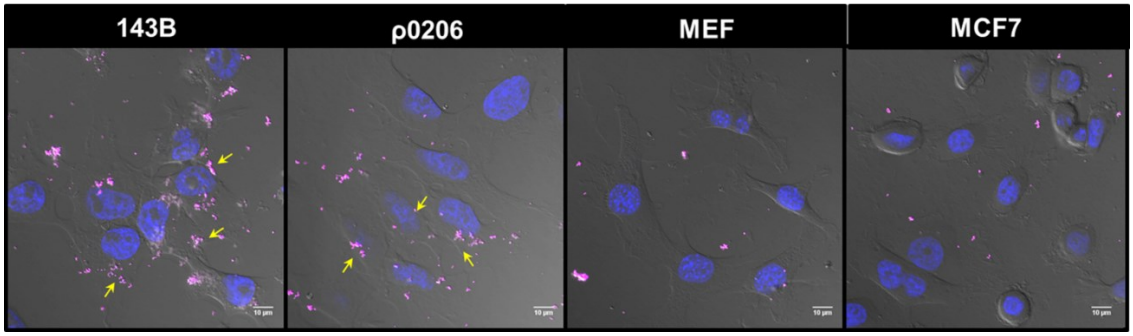


Figure S9. Selective nanofection of CD147-Cy5-NPs (**7B-2**) by confocal microscopy. Confocal microscope images of the cellular uptake behavior of CD147-Cy5-NPs (**7B-2**) in 143B, p°206, MEF and MCF7 cell lines. 63X magnification representative images show: merge, DIC (Differential interference contrast), blue, DAPI nuclei and magenta CD147-Cy5-NPs (**7B-2**) overlaid. Scale bar, 10 µm.

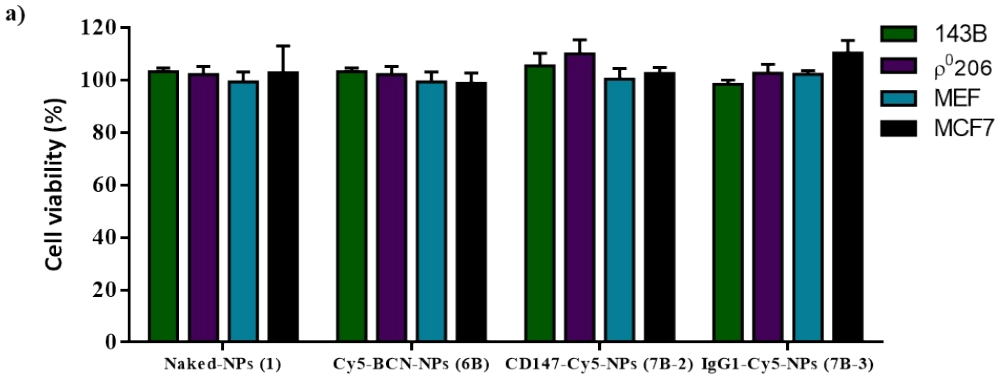


Figure S10. Viability of 143B, p°206, MEF and MCF7 cell lines by resazurin cell viability assay after the internalization of a) Cy5-BCN-NPs (**6B**), CD147- Cy5-NPs (**7B-2**) and IgG1-Cy5-NP (**7B-3**) compared to Naked-NPs (**1**).

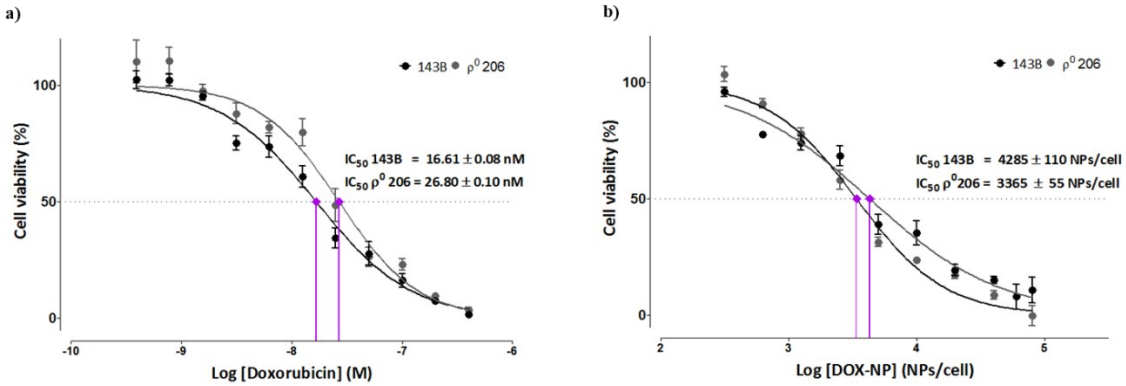


Figure S11. Dose-response curves (percentage of cell viability versus concentration) of treatment of 143B AND p°206 cancer cells with: a) free DOX, represented in M; b) CD147-DOX-NPs (**11-2**), represented in NPs/Cell. The IC50 value was determined by the logarithm (inhibitor) versus normalized response: variable slope using the GraphPad software.

2. General experimental methods

2.1. General information

Chemistry equipment:

The HPLC analysis was performed on an Agilent 1200 series HPLC system coupled to a PL-ELS 1000 evaporative light scattering detector (ELS) from Polymer Lab with UV detection at 220, 254, 260, 282 and 495 nm, Discovery® C 18 of Supelco (50 mm x 2.1 mm x 5 µm). The elution was carried out with Solvent A (0.1% formic acid in deionized HPLC grade water) and Solvent B (0.1% formic acid in HPLC grade methanol) at 1 ml x min with a gradient of 5 to 95% B for 3 minutes, followed by 1 minute isocratic to 95% of B and ending with a gradient of 95 to 5% of B for 1 minute, then 1 min of isocratic to 95% of A.

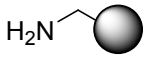
All conjugations were carried out with an Eppendorf Thermomixer® agitator and the centrifugations were performed in an Eppendorf centrifuge.

Biology equipment:

Cell cultures were performed in a NU-4750E US AutoFlow incubator from NUAIRE.

Cell experiments were carried out in a laminar flow cabinet Bio II A of TELSTAR Class II A.

2.2. Synthesis of cross-linked polystyrene aminomethyl NPs (Naked-NPs (1))



1

PVP (Mw 29,000, 0.05g, 1.7 µmol, Sigma-Aldrich) was dissolved in 92% ethanol/8% water for a final volume of 10 mL, and deoxygenated via argon bubbling. AIBN (7 mg, 42.4 µmol) was dissolved in styrene (freshly washed, 0.5 mL) with VBAH (7 mg, 41.3 µmol) and DVB (freshly washed, 4.65 µL) [2]. The dispersion was deoxygenated with argon bubbling before addition to the PVP/Ethanol solution.

The mixture was stirred under argon for 1 hour before heating to 68 °C for 15 hours. NPs were obtained by centrifugation (11,000 G, 15 minutes) and washed with methanol (2 x 10 mL) and water (2 x 10 mL). Finally, NPs were stored in water (10 mL) at 4°C.

Particle size distribution: mean diameter: 439.2 nm, PDI: 0.03.

Loading (Ninhydrin): 0.069 mmol / g.

Number of particles per gram: 1.53×10^{13}.

2.2.1. Characterization of Naked-NPs (1)

2.2.1.1. Solid content (SC) of the emulsion (%)

A known mass of a suspension of polystyrene NPs (0.5-1mg, suspended in water) was placed in a watch glass, covered with aluminium foil, dried at 25 °C for 15 hours, weighed and reweighed to give the mass of NPs. The solid content was then calculated according to the following equation:

$$\% \, SC = \frac{M}{Vs} \, x \, 100$$

Where m = mass of NPs (mg), Vs = Volume of suspension (µL).

CS: 2%, 2 mg of NPs in 100 µL of solution.

2.2.1.2. Calculation of number of particles per gram

$$N = {}^{6x10^{12}}\!/_{\pi x \rho x d^3}$$

Where N = Number of particles/g for dry powder, ρs= Density of solid spheres (g/cm^3), which is 1 g/cm^3 for polystyrene, d= Mean diameter (nm).

Result: N= 1.53 x 10^{13} NPs per gram.

2.2.1.3. Calculation of loading of NPs using Fmoc NPs test

Fmoc-(x)-NPs (where x is Fmoc-PEG-OH or Fmoc-Lys(Dde)-OH, etc) were resuspended in 1 mL of 20% piperidine in DMF (3 x 20 min) after which the beads were washed by centrifugation three times, the supernatants combined and the loading was calculated according to the following equation:

$$Loading \left({}^{mmol}\!/_{g} \right) = \frac{(A_{302} x V)}{(\varepsilon_{302} x d x W)} x 1000$$

Where A_{302} : Absorbance measured at 302 nm, V_{mL} : Volume of combined supernatants, ε_{302} : Molar Extinction Coefficient (7800 $M^{-1}cm^{-1}$) and W_{mg} : Mass of beads.

Result: Loading (Fmoc test): 0.069 mmol/g

2.2.1.4. Qualitative ninhydrin test

The reaction control was determined by qualitative ninhydrin test. Small samples of NPs in methanol (12 µL, 2% SC) in a 0.5 mL capacity eppendorf were washed with methanol and centrifuged after which 6 µL of reagent A and 2 µL of reagent B were added. Mix well and heat to 100 ºC for 3 min. Blue stained resin beads indicate the presence of primary amines.

2.3. Determination of NPs concentration (NPs/µL) by spectrophotometric method

NPs concentration (NPs per microliter) was determined by a spectrophotometric method as described previously[3]. Briefly, measurement of turbidity optical density at 600 nm of polystyrene NP suspensions was performed, based on nephelometric principals. Light going through NP suspensions is scattered via reflection, refraction and diffraction phenomena and the intensity of the scattered light, which are proportional to number of NPs in suspension, is recorded by standard spectrophotometers. In this way, calibrate standard curves were obtained for amino-methyl cross-linked polystyrene NPs of 440 nm by NP known concentrations. Calibration curves fitted linear regression models by which the number of NPs per microliter corresponding to one unit of OD600 for each size could be determined. Thus, these curves using initial batches of NP suspensions permitted us to estimate the number of NPs in final batches, which underwent multiple handling procedures, by OD600 measurement of 1 µL (**Figure S6**).

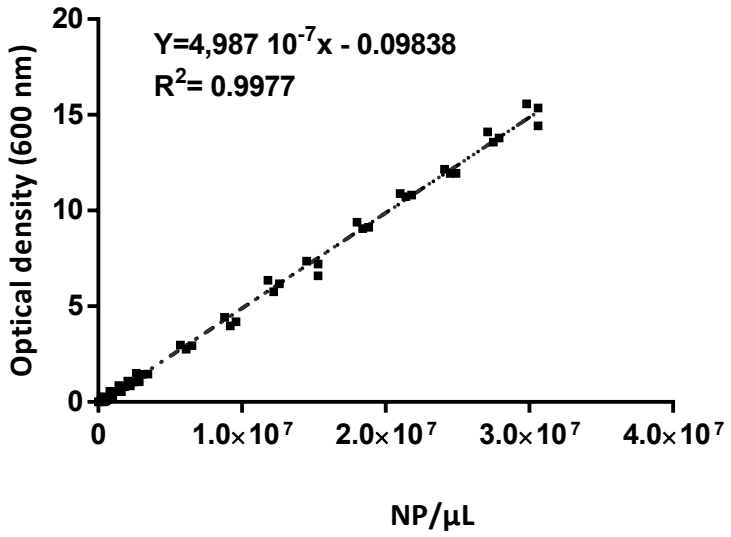


Figure S11. Calibration standard curve of concentration of nanoparticles (OD 600)

2.4. General protocol for Fmoc and Dde deprotection

2.4.1. Fmoc deprotection

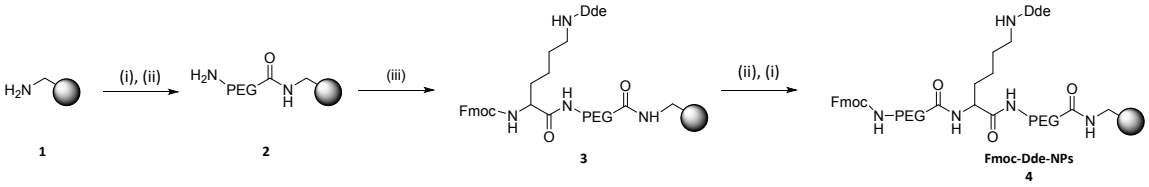
Fmoc deprotection was achieved by treating NPs with 20% piperidine/DMF (1 mL; 3 x 20 min). NPs were obtained by centrifugation and subseq.uently washed with DMF (3 x 1 mL), MeOH (3 x 1 mL), deionised water (3 x 1 mL).

2.4.2. Dde deprotection

Dde deprotection was facilitated by treating NPs with the Dde deprotection solution mixture (1.25 g (1.80 mmol) of $NH_2OH.HCl$ and 0.918 g (1.35 mmol) of imidazole were suspended in 5 mL of NMP, and the mixture was sonicated until complete dissolution. Just before reaction, 5 volumes of this solution were diluted with 1 volume of DMF (1 mL) for 1 hour at r.t. on a rotary wheel, then NPs were washed with DMF (1 mL). NPs were obtained by centrifugation and subsequently washed with DMF (3 x 1 mL), methanol (3 x 1 mL), deionised water (3 x 1 mL) and finally DMF (3 x 1 mL).

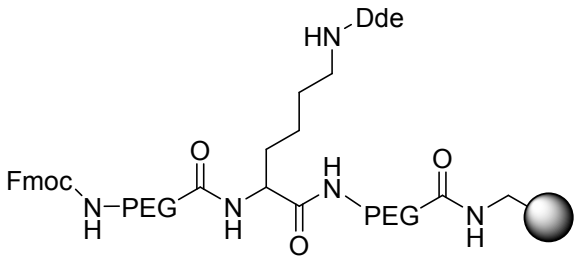
3. Preparation of bifunctionalized Fmoc-Dde-NPs (4)

3.1. General scheme for synthetic strategy to obtain Fmoc-Dde-NPs (4)



Scheme S1. General scheme of synthesis of Fmoc-Dde-NPs (4). Reagents and conditions: (i) Fmoc-PEG-OH (15 eq.), Oxyma (15 eq.), DIC (15 eq.), DMF. 2 h, 60ºC; (ii) 20% piperidine, DMF. 3 x 20 min; (iii) Fmoc-Lys(Dde)-OH,Oxyma (15 eq.), DIC (15eq.), DMF. 2 h, 60ºC; (iv) BCN (10 eq.), DIPEA (1 eq.), DMF. 15 h, 25ºC; (v) Hydroxylamine.HCl, Imidazole, NMP, 2x 1 h, 25ºC.

3.2. Preparation of Fmoc-Dde-NPs (4)



Fmoc-Dde-NPs
4

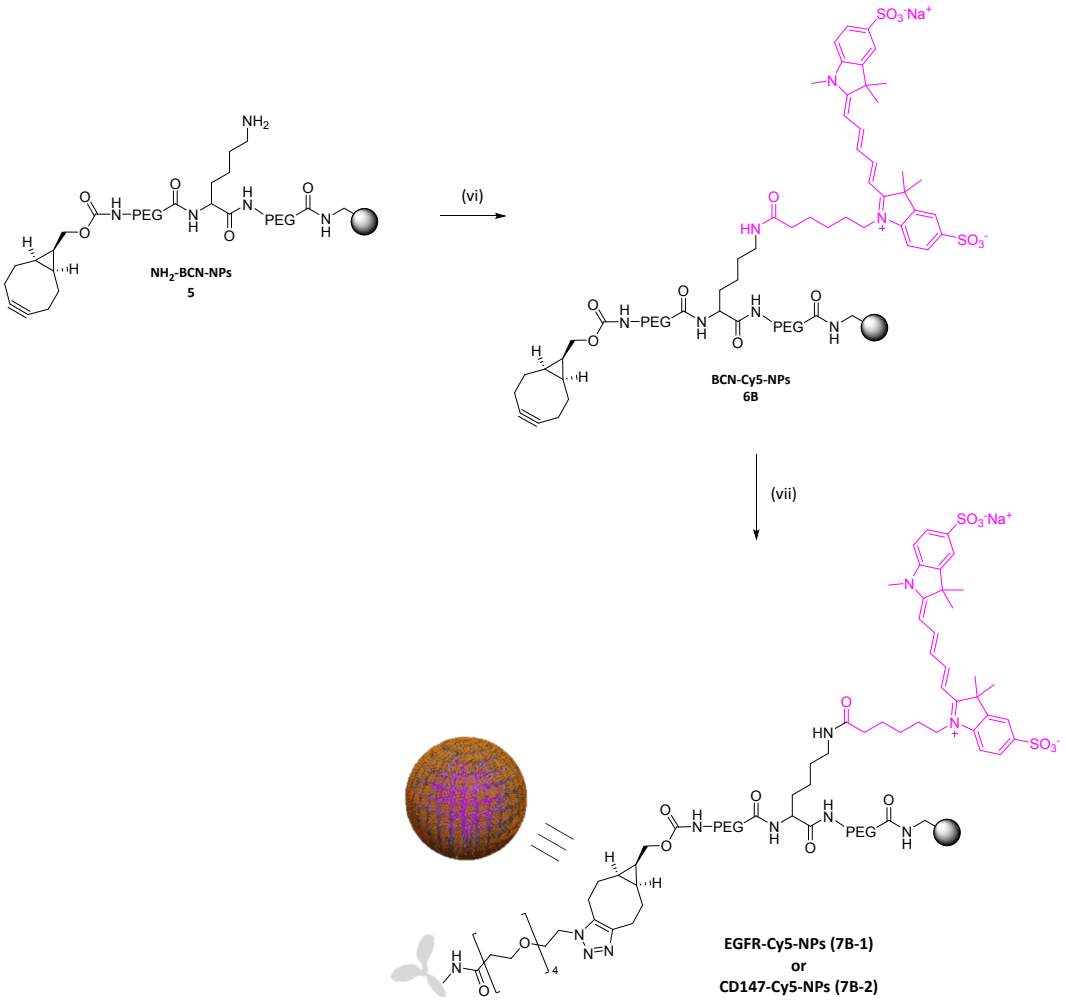
Aminomethyl NPs **1** (1 mL, 2% SC, 54 µmol/g, 1 µmol, 1 eq.) were washed in DMF (1 mL x 3 times) and suspended in DMF (1 mL). Separately, the Fmoc-PEG spacer (15 eq.) was dissolved in DMF (1 mL), then oxyma (15 eq.) was added and the solution mixture mixed for 4 minutes at r.t. before the addition of DIC (15 eq.) and mixed for 8-10 minutes at 25ºC. The solution

mixture was then added to amino NPs and suspension mixed on the Thermomixer at 1400 rpm for 2 hours at 60°C [4]. Fmoc deprotection was achieved by treating NPs with 20% piperidine/DMF.

PEG functionalised NPs **2** (1 mL; 1 eq.) were washed in DMF (1 mL x 3 times) and suspended in DMF (1 mL). Separately, Fmoc-Lys(Dde)OH (15 eq.) was dissolved in DMF (1 mL), then oxyma (15 eq.) was added and the solution mixture mixed for 4 minutes at r.t. before the addition of DIC (15 eq.) and mixed for 8-10 minutes at r.t.[5] The solution mixture was then added to NPs **2** and suspension mixed on the Thermomixer at 1400 rpm for 2 hours at 60°C. Then this step was repeated to Fmoc deprotection and to introduce one unit PEG spacer to obtain Fmoc-Dde-NPs (**4**).

4. Preparation of Ab-NPs

4.1. General scheme for synthetic strategy to obtain EGFR-Cy5-NPs (7B-1) and CD147-Cy5-NPs (7B-2)



Scheme S2. General scheme of synthesis of fluorescent EGFR-Cy5-NPs (7B-1). Reagents and conditions: (vi) Sulfo-Cy5-NHS (1 eq.), DIPEA (0,1 eq.), 15 h, 25ºC; (vii) Ab-N_3, PBS, 15h, 25ºC.

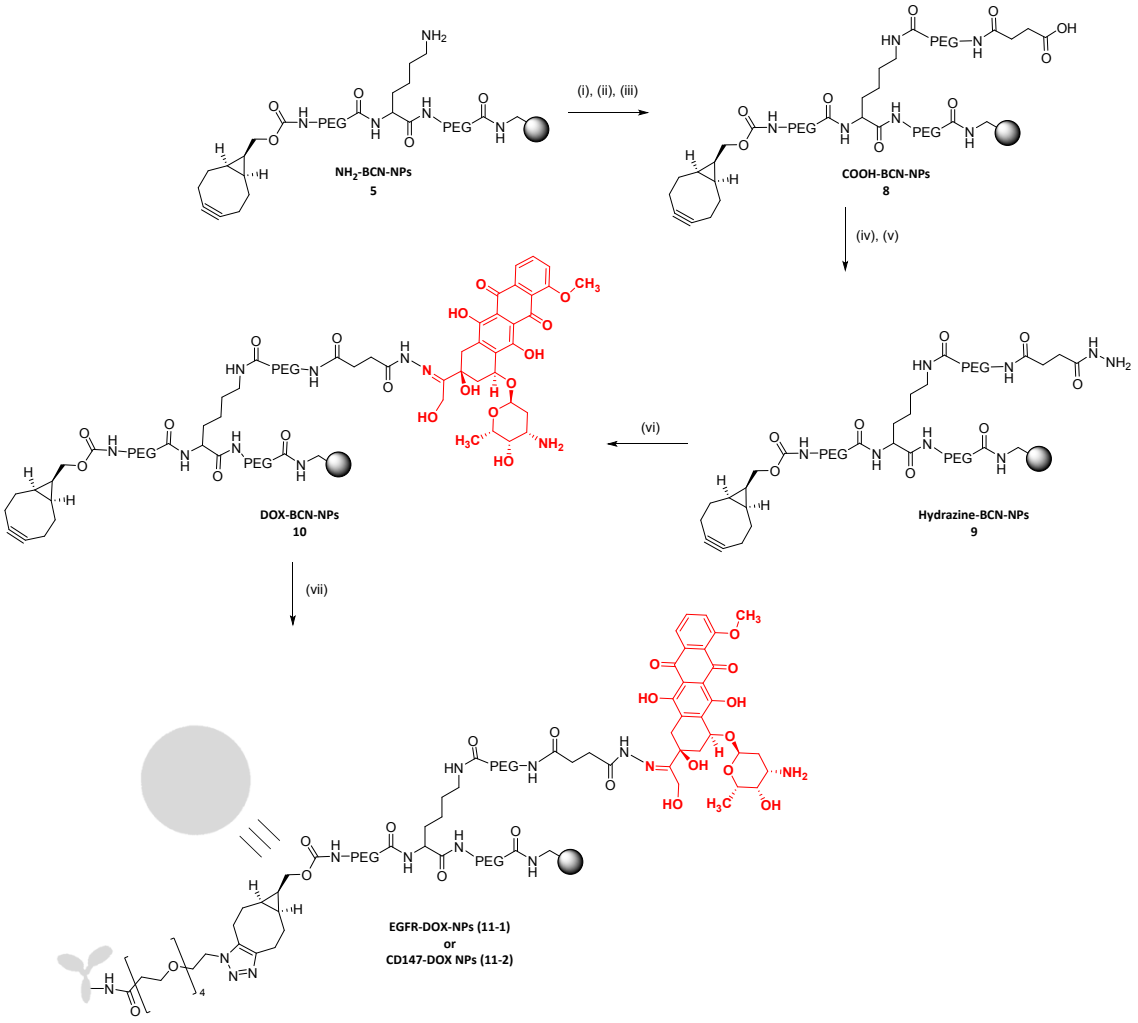
Then labelled of NPs was carried out, NPs (1mL, 1 eq.) were washed (3 x 1 mL) and resuspended in anhydrous DMF (1mL). Sulfo-Cy5-NHS (1 eq.) was dissolved in anhydrous DMF (1mL) and DIPEA (1 eq.) and suspension mixed on the Thermomixer at 1400 rpm for 15 hours at 25 ºC. The solution mixture was then added to NPs and suspension mixed on the Thermomixer at 1400 rpm for 2 hours at 60°C. NPs were washed and obtained Cy5-BCN-NPs (**6B**).

The conjugation NPs with anti-Ab (EGFR or CD147) functionalized with azide group was optimized for 3 x 10^{10} NPs (quantified by the spectrophotometric method) and 2.5 µM of antibody dissolved in PBS and suspension mixed on the Thermomixer at 1000 rpm for 12 hours at 25 ºC. Reaction finished, NPs were washed with sterile PBS, the first supernatant was saved for BCA quantification, and EGFR-Cy5-NPs (**7B-1**) or CD147-Cy5-NPs (**7B-2**) were obtained.

The **irreverent IgG1** antibody was conjugated using the same functionalization and conjugation process followed with the anti-IgG1 antibody, finally obtaining IgG1-NP (**7B-3**).

5. Preparation of Ab-DOX-NPs (11)

5.1. General scheme for synthetic strategy to obtain Ab-DOX-NPs (11)



Scheme S4. General scheme of synthesis of Ab-DOX-NPs (**11**)**.** Reagents and conditions: (i) Fmoc-PEG-OH (15 eq), Oxyma (15 eq), DIC (15 eq), DMF, 2 h, 60 ° C; (ii) 20% piperidine/DMF, 3 x 20 min; (iii) Succinic anhydride (15 eq), DIPEA (15 eq), 2 h, 60 °C; (iv) Oxyma (15 eq), DIC (15 eq), 2 h, 25 °C; (v) Hydrated hydrazine 55% v/v (15 eq), 15 h, 25 ° C; (vi) Doxorubicin (1 eq), PBS pH 6, 15 h, 50 ° C. (vii) Ab-N_3, PBS, 15h, 25ºC.

Firstly, the conjugation of the succinic anhydride (15 eq.) with DIPEA (7.5 eq.) was carried out to obtain COOH-BCN-NPs (**8**). Later, NPs (**8**) were activated with oxyma (15 eq.) and DIC (15 eq.) during 4 hours and they were centrifuged and a solution of 55% v/v hydrazine in DMF was added and they were left stirring for 15 hours at 25 ° C. Next, the Hydrazine-BCN-NPs (**9**) were washed and conditioned in 1 mL of PBS pH 6. Then, 1 equivalent of DOX was dissolved in PBS at pH 6 and added to the NPs and the resulting mixture was mixed for 15 hours at 50 °C to yield DOX-BCN-NPs (**10**). Finally, the conjugation of anti-EGFR or anti-CD147 with DOX-BCN-NPs (**10**) was carried out to obtain EGFR-DOX-NPs (**11-1**) or CD147-DOX-NPs (**11-2**).

6. Characterization of Ab-NPs (7) and Ab-DOX-NPs (11).

6.1.1. Determination of fluorophore concentration

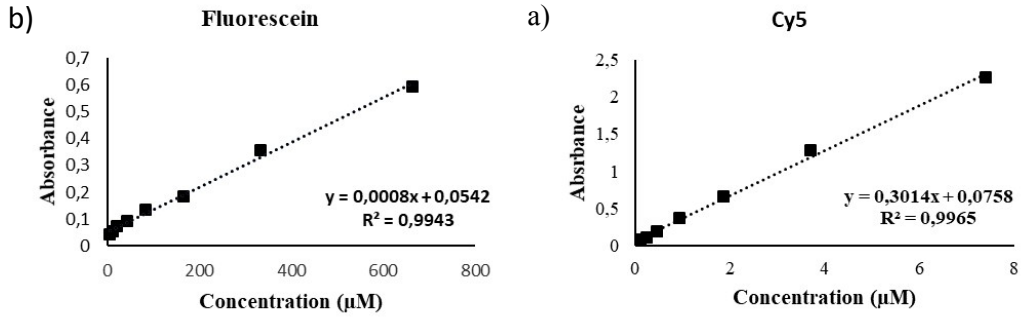


Figure S12. Calibration standard curve of fluorophores (Fluorescein and Cy5) by spectrophometry.

6.1.2. Determination of antibody concentration using BCA assay

The conjugation efficiency was determined by measuring the concentration of antibody (EGFR and CD147) present in the supernatants once the coupling reaction was completed. The absorbance values obtained at 562 nm were translated into concentration using a standard calibration curve (**Figure S13**). [6] The results show a conjugation efficiency of 100%, as no antibody was found in the supernatants (**Table S.3-4**).

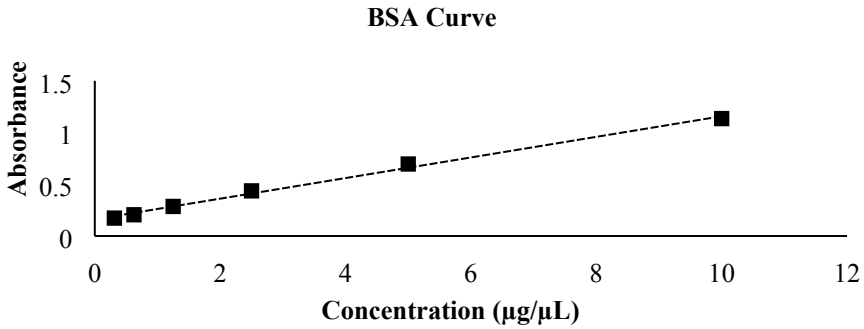


Figure S13. BSA calibration curve for antibody quantification.

Table S3. Calculation of anti-EGFR concentration using BCA assay

NPs	Absorbance	Concentration (mg/mL)	Conjugation efficiency (%)
antiEGFR-N_3	0,535	1,873326689	-
EGFR-F-NPs (**7A**-**1**)	0,071	-0,456827305	100
EGFR-Cy5-NPs (**7B**-**1**)	0,044	-1,181392223	100
EGFR-DOX-NP (**11**-**1**)	0,141	-0,214524744	100

Table S4. Calculation of anti-CD147 concentration using BCA assay

NPs	Absorbance	Concentration (mg/mL)	Conjugation efficiency (%)
antiCD147-N_3	0,413	1,5220884	-
CD147-Cy5-NPs (**7B-2**)	0,098	-0,642570249	100
CD147-DOX-NP (**11-B**)	0,1273	-0,347054889	100

6.1.3. Agarose gel

15 µL of Ab-NPs (**7A-1**, **7B-1**, **7B-2**, **11-1, 11-2**) were loaded with a 1: 5 dilution of loading buffer and 1 µg of functionalised anti-EGFR antibody in an agarose gel at 0.8%. Electrophoresis was performed in a TAE solution at pH 8.5 for 30 minutes at 90 mV. [7,8] Subsequently, the gel was stained with a Coomassie blue solution.[9]

6.1.4. Immunofluorescence.

EGFR-NPs (**7A-1, 7B-1, 11-1**) were resuspended in a blocking solution and allowed to stir (Ferris wheel) for two hours at 4 °C. They were then washed twice with PBS and resuspended with a 1: 100 solution of Rabbit anti-Mouse IgG secondary antibody, Alexa488 (Thermo Fisher) for EGFR-Cy5-NPs (**7B-1**), CD147-Cy5-NPs (**7B-2**) and Ab-DOX-NPs (**11**), and Goat anti-Mouse IgG secondary antibody, Alexa647 (Thermo Fisher) for EGFR-F-NPs (**7A-1**), and incubated for 30 minutes with shaking (Ferris wheel) and darkness. Finally, they were washed twice with PBS.[10]

6.1.5. Conjugation, release profile and calibration curve of doxorubicin by HPLC method of EGFR-DOX-NPs (11-1) and CD147-DOX-NPs (11-2)

To determine the amount of DOX loaded on the nanoparticle surface (Loading Capacity, LC) and to evaluate the efficiency of the conjugation process (Conjugation Efficiency, CE), the concentration of free DOX in the supernatant obtained after the centrifugation of NPs was measured by UV spectroscopy at 480 nm. A calibration curve, with lineal ratio between the optic density of DOX and its concentration, was previously performed (**Figure S14a**). Then, DOX loading capacity (LC) and DOX conjugation efficiency (CE %) was calculated according to formulas as follows:

$$LC = \frac{[DOX\ \ conjugated\ on\ surface\ of\ nanoparticle]}{Number\ of\ NPs} \times N_A$$

Where N_A is Avogadro's number.

$$CE(\%) = \frac{[DOX\ conjugated\ on\ surface\ of\ nanoparticle]}{Total\ concentration\ of\ DOX\ added} \times 100$$

The conjugation efficiency of DOX conjugated covalently was 90.2% and 93.7% with a concentration of 6.9 x 10^{-9} nmol DOX / NP and 3.31 x 10^{-9} nmol DOX / NP to EGFR-DOX-NPs (**11-1**) and CD147-DOX-NPs (**11-2**), respectively.

Cumulative release was determined using this equation:

$$(\%) = \frac{D_t}{D_T} \times 100$$

Where, Dt is the concentration of DOX released from DOX-EGFR-NPs (**11-1**) and CD147-DOX-NPs (**11-2**) at time t and DT is the concentration of DOX-loaded onto the DOX-EGFR-NPs (**11-1**) and CD147-DOX-NPs (**11-2**) (**Figure S14 b-c**).[11]

Analysis of the efficacy of the release of doxorubicin by HPLC: chromatographic conditions: the HPLC system (Acquity UPLC System, Waters) with a C18 column from Waters CORTECS ™ (2.1 mm x 75 mm, 2.7 µm). The detection of PDA eλ for doxorubicin was established at 252 nm. The mobile phase of water: acetonitrile (30:70, pH 3.0, adjusted with 85% phosphoric acid) was supplied at a flow rate of 0.4 ml / min of acetonitrile: 0% B, T8: 95% B, T8.1: 0% B, analysis time 10 minutes. The maximum identification was confirmed by the retention time (RT) of doxorubicin hydrochloride at 2.69 min (**Figure 14d**).

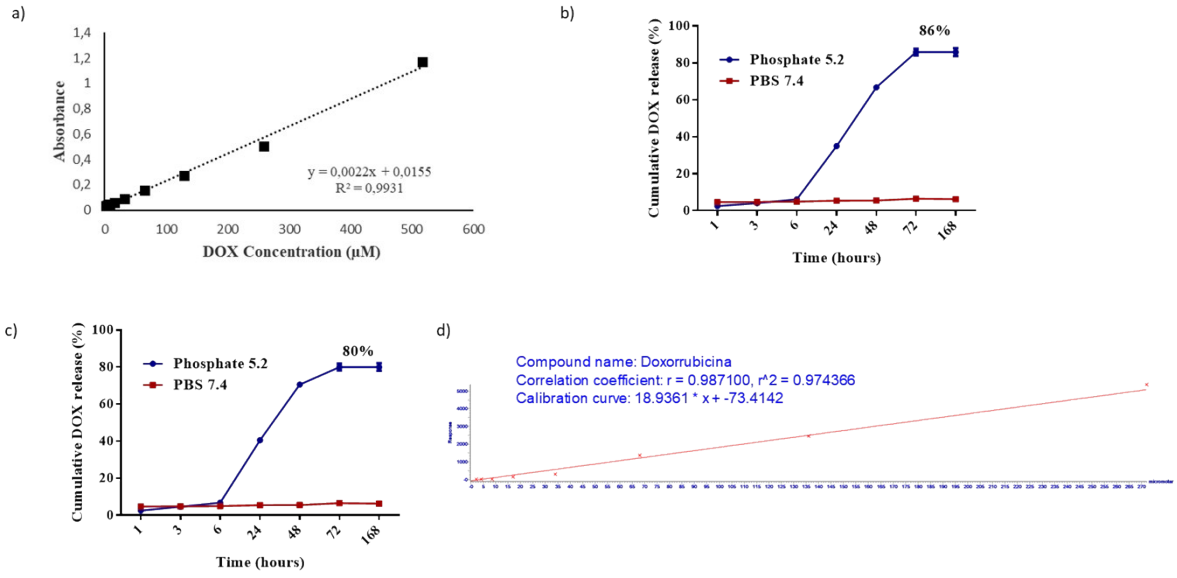


Figure S14. a) Calibration curve for DOX quantification; b) Release profile of DOX from EGFR-DOX-NPs (**11-1**); c) Release profile of DOX from CD147-DOX-NPs (**11-2**); d) Calibration curve of DOX obtained by HPLC.

7. General protocol for cellular nanofection

7.1. Cellular nanofection by flow cytometry

After incubation with NPs, the medium was aspirated and the cells were washed with 1x PBS and separated with trypsin-EDTA at 37 °C for 5 minutes. Then, each sample was fixed in 2% paraformaldehyde (PFA) at room temperature for 10 minutes and protected from light. Samples were analyzed by flow cytometry with a FACSCanto II flow cytometer. Each experiment was performed in duplicate by ratio and time of incubation and was repeated at least three times.

The study of the nanofection of EGFR-F-NPs (**7A-1**) and control NPs were carried out using the following ratio of NPs per cell: 1/50; 1/100; 1/250; 1/500; 1/1000; 1/2500; 1/5000; 1/10000. The dot plots and the cytometry stadistics were obtained using the FlowJo software. Graphs and statistical difference data were made using the GraphPad software according to the following explanation. The percentages of cell data containing NPs were plotted against the cell / NPs ratio (3.3) in a bar representation to establish the statistically significant differences mediated by the analysis of Student's t-test in paired groups of samples comparing the same treatments between different cells lines.

7.2. Cellular nanofection by confocal microscopy

The cells were washed with 1X PBS, separated with trypsin / EDTA, counted and diluted with the corresponding media to a final concentration of 10^5 cells per mL. 500 µL of each cell line suspension was seeded onto glass coverslips coated with poly-L-lysine in 24-well plates and incubated for 15 h. Then, the media was replaced by a new solution with the medium containing the quantity of NPs corresponding to each experiment. After the corresponding incubation time, the medium was aspirated and the cells were washed with 1X PBS and fixed in 4% paraformaldehyde at room temperature for 30 minutes. The fixed cells were washed with 1X PBS and mounted with ProLong Gold mounting medium with DAPI (Life technologies). The images were collected with the ZEISS LSM 710 confocal laser microscope using a DIC Plan-Apochromat 63x oil immersion objective with 1.40 numerical aperture and the ZEN 2010 software. The image analysis was subsequently carried out with the ZEN 2012 program Blue Edition or the program ImageJ version 1.49b (free software). Samples containing fluorescent NPs and DAPI nuclear staining were excited using a HeNe laser line of 633 nm wavelength (5.0 mW) and 7% power for the NPs, a laser line of 405 nm diode wavelength (30.0 mW) 2.8% power for DAPI and HeNe laser line with wavelength of 543 nm (1.2 mW) 20% power for DIC

images and 1.00 Airy unit (AU). Each experiment was performed in duplicate and repeated three times per cell line.

8. Cell viability

The cellular cytotoxicity of NPs was evaluated using resazurin assay protocol. Cells were seeded in 96-well plates at a density of 1 x 103 cells for A549 and 3 x 103 for H520 MCF7, MEF, 143B and ρ0206 per well and incubated for 15 hours. Then, the medium was replaced with fresh medium containing the tested samples at various concentrations. After incubation for 96 hours, the medium containing NPs was removed and replaced with phenol red free fresh complete medium. The results were evaluated according to the manufacturer's protocol, and the amount of fluorescence obtained was proportional to the number of viable cells. Viability was expressed with respect to the percentage of untreated cells (100%). Control wells were included in each plate to measure the fluorescence of the culture medium with NPs added in the absence of cells.

9. Determination of DNA damage in cancer cells by inmunostaining of phospho-H2A.X foci.

A549 and H520 cells were cultured in DMEM supplemented with 10% FBS, L-glutamine and penicillin/streptomycin on coverslips in 24-well plates. Cells were incubated with 5,000 EGFR-DOX-NPs (11-1) per cell. After 24 hours of incubation, the media was replaced with fresh full DMEM. 1 h after change of medium the cells were fixed with 4% PFA for 10 minutes at room temperature. After fixation, the cells were washed with PBS and incubated with blocking buffer containing 5% BSA and 0.3% Triton X-100 in PBS for 1 hour at room temperature. Then, cells were incubated with a solution 1:500 of primary antiphospho-H2A.X antibodies in blocking buffer (Cell Signalling, 20E3, 1:500) at +4°C overnight (300 µL/well). The next day, cells were washed with PBS and stained with a 1:1000 solution of secondary Alexa Fluor 488 conjugated antibodies (Invitrogen, A-11034) for 1 h at room temperature. After washing with PBS, the preparations were mounted with mounting medium including antifade and DAPI (Invitrogen). [12] For automatic H2A.X foci counting, ImageJ® analysis was performed.

10. RNA extraction, cDNA synthesis and quantitative PCR

Total RNA from human cells was isolated using the miRNeasy Extraction Kit (Qiagen) and quantified using a NanoDrop 2000 spectrophotometer (Thermo). When necessary, the quality

of the RNA was evaluated by electrophoresis, loading 200 ng in a 2% agarose gel in TAE buffer (tris base, andagnésic acid EDTA). Single-stranded cDNA synthesis was performed using oligo dT-adapter primers and the Transcriptor First Strand cDNA synthesis kit (Roche) according to the manufacturer's protocols, starting from 500 to 1000 ng of original RNA. The cDNA was amplified with gene specific primers and Fast Sybr Green in the 7900HT system (Applied Biosystems). In each trial, we used 30 ng of cDNA per reaction, including controls without template. Each sample was processed in triplicate in each experiment and each experiment was repeated at least three times. The expression of mRNA in each sample was normalized for the endogenous maintenance gene β-actin. The relative expression of the mRNA was analyzed using the 2-ΔΔCt method as previously described [13]. The error bars show the standard error of the mean (SEM).

To determine the expression levels of the EFG and CD147 receptors in several cell lines, a qPCR (quantitative polymerase chain reaction) was performed (**Figure S15**).

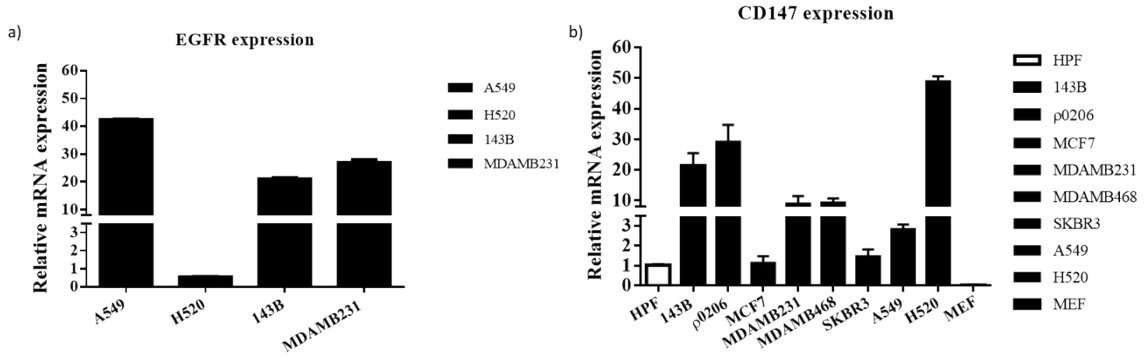


Figure S15. Representation of the quantitative PCR analysis (value 2 -ΔΔCt) of the genetic expression of a) EGFR and b) CD147 in several cell lines.

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